3i Intelligent Imaging Innovations

AxL Cleared Tissue LightSheet

Axially Swept Light Sheet Microscopy System for Imaging Cleared Specimens

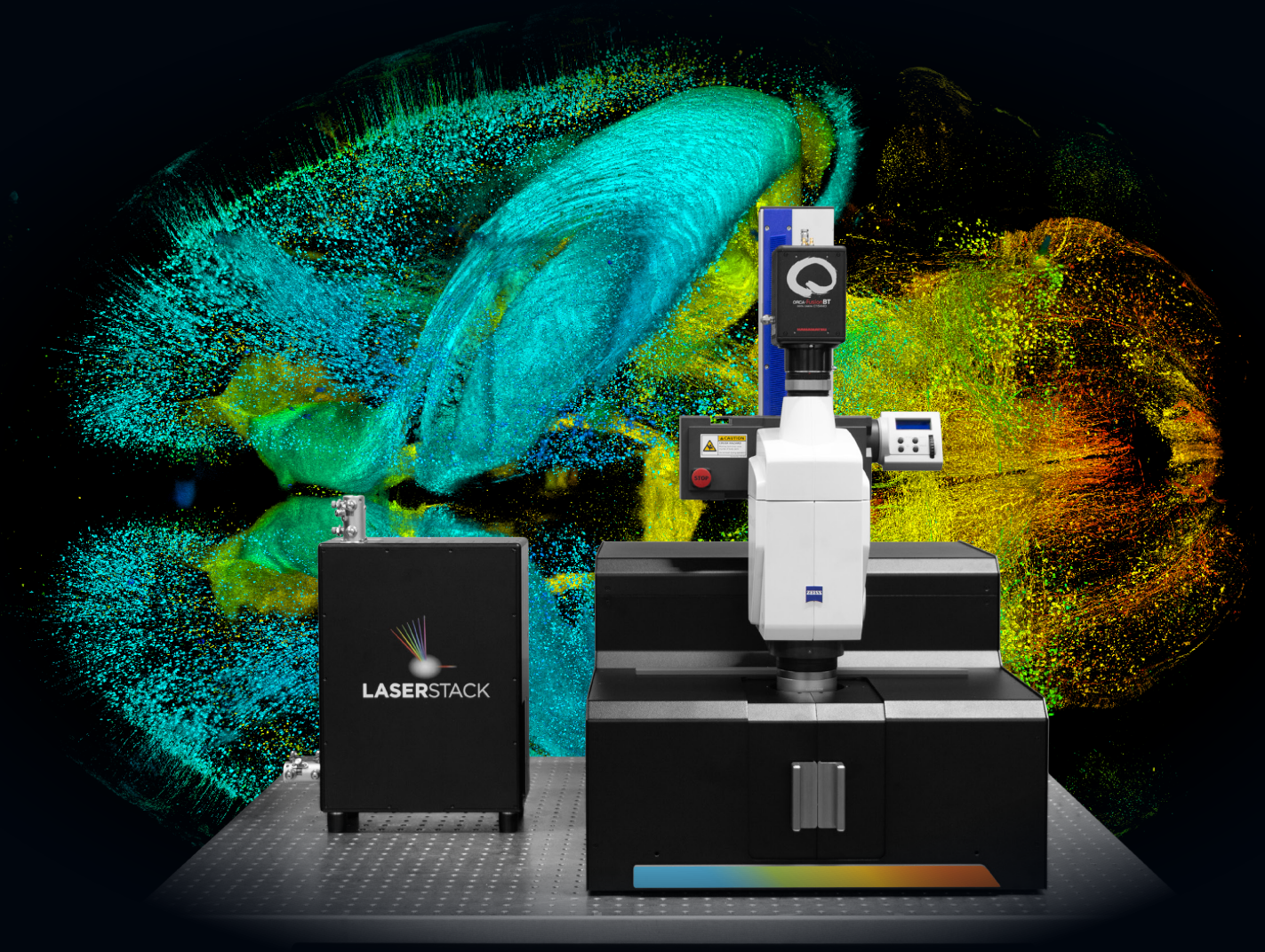


Image Courtesy of Dr. H. Zhao, Beijing Institute for Brain Research.

AxL Cleared Tissue LightSheet

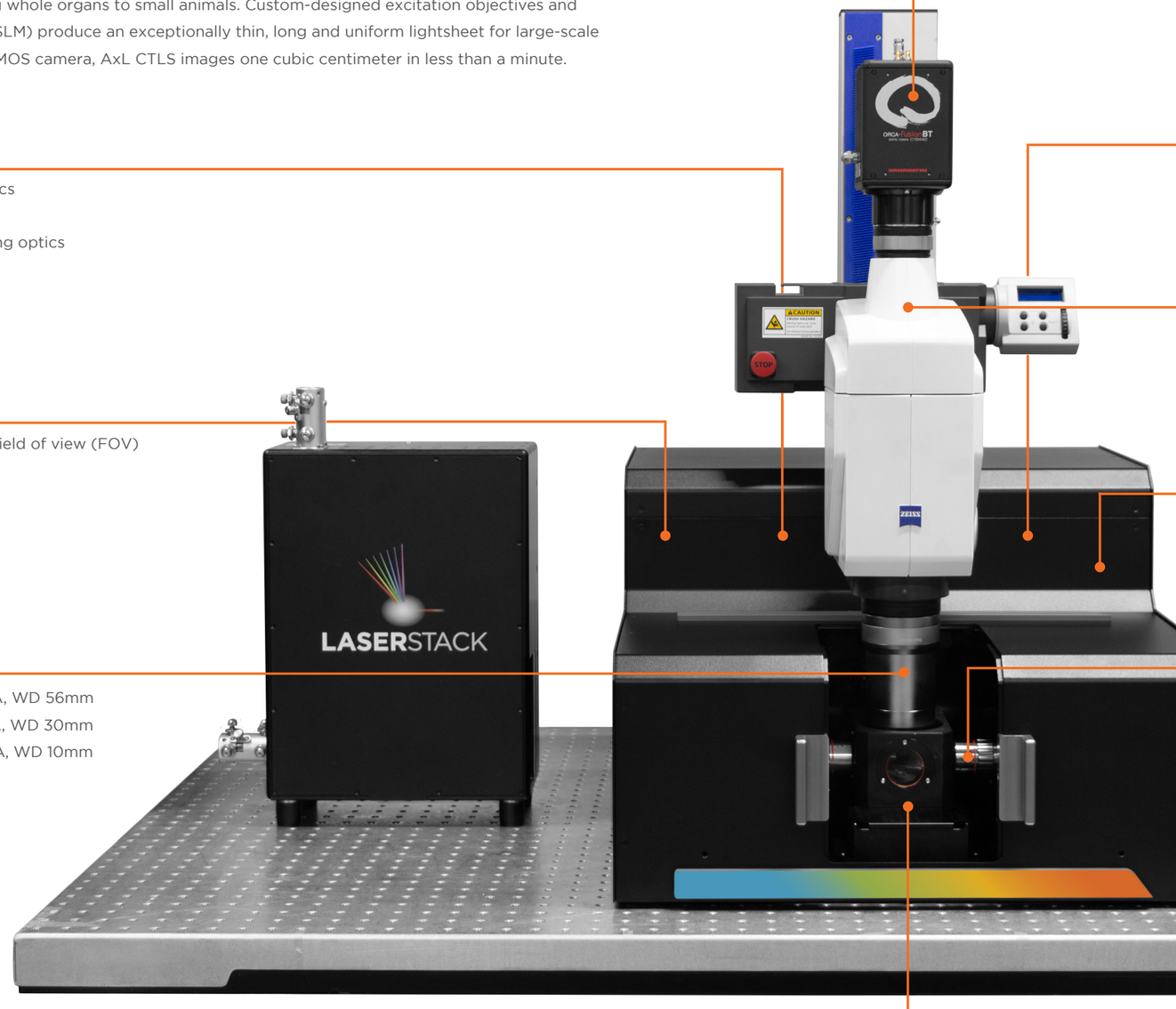
AxL Cleared Tissue LightSheet (AxL CTLS) is a fully automated macro zoom microscope with high NA apochromatic objectives and dual-sided light sheet illumination for imaging whole organs to small animals. Custom-designed excitation objectives and patented axially swept light sheet microscopy (ASLM) produce an exceptionally thin, long and uniform lightsheet for large-scale high-resolution imaging. Using a back-thinned sCMOS camera, AxL CTLS images one cubic centimeter in less than a minute.

AxL CTLS Scanner

Proprietary dual-sided light sheet illumination optics
Compact 54 x 60cm footprint
Seamlessly switch imaging modes without changing optics
- Axially swept light sheet mode
- Ultrafast 3D prescan mode

Electrically Tunable Lens

Axial light sheet sweep length of 10mm for large field of view (FOV)



Back-thinned sCMOS Camera

2304 x 2304 6.5µm pixels
95% quantum efficiency
Rolling shutter mode

Multichannel Z Galvo

High-speed chromatic aberration correction

Automated Zoom Microscope

Motorized 16:1 zoom for optimal pixel sampling

Dynamic Galvo-Scanned Light Sheet

Thin and uniform sheet across a 10mm FOV
Adjustable FOV for faster imaging of smaller specimens

Macro Fluorescence Objectives

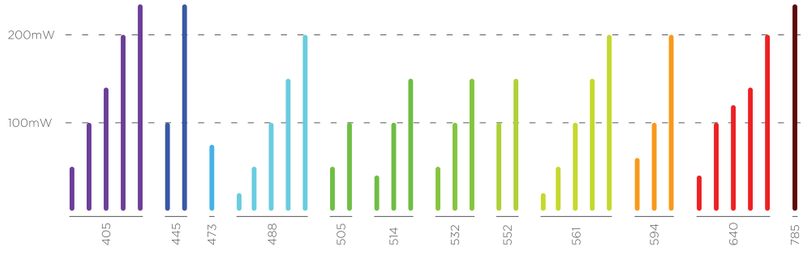
1.0x/0.25NA, WD 56mm
1.5x/0.37NA, WD 30mm
2.3x/0.57NA, WD 10mm

Custom Excitation Objectives

Spherical aberration correction for high-refractive-index immersion media
Flat excitation profile across 1mm to 10mm sheet width
0.14NA diffraction-limited light sheet

LaserStack Laser Combiner

Modular Laser Combiner
Up to 8 lasers
Up to 300mW

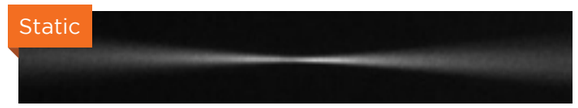


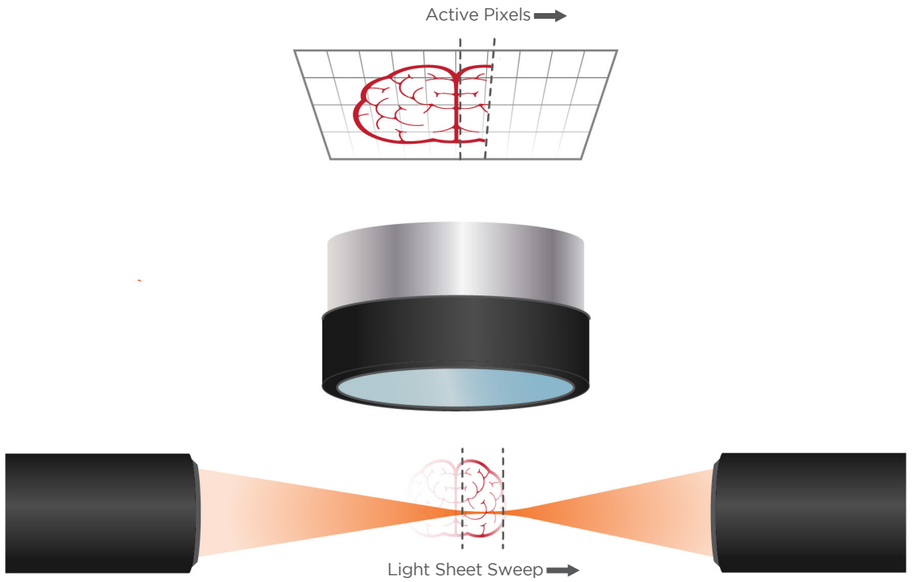
Chambers and Specimen Holders

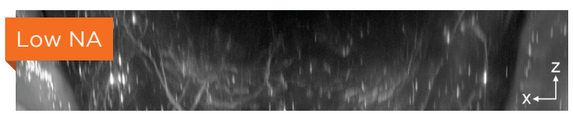
Sample chambers in 4 different sizes
Temperature controlled
Chemically-inert sample holders
Clearing solutions ranging from 1.33-1.56 RI
Multi-specimen holder for up to 10 different samples

Axially Swept Lightsheet Microscopy

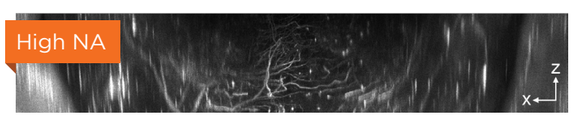
Axially swept lightsheet microscopy (ASLM) - as published in Dean, Fiolka et al., 2015 - scans the light sheet in its propagation direction using high-speed remote focusing synchronized to the rolling shutter readout of an sCMOS camera at the size of the beam waist. This approach creates an exceptionally thin light sheet across a large field of view resulting in images with improved optical sectioning and signal-to-noise. This scanned sheet features a constant laser intensity across the field of view for an evenly illuminated image. The 0.14NA excitation objective creates a 2µm thin waist for superior axial resolution. AxL CTLS is designed to operate optimally across a range of 1.33 to 1.56 refractive indices ensuring compatibility across all available clearing methods.



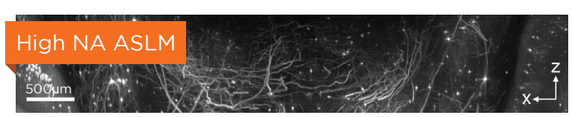




Typical light sheet illumination across the entire field of view.



Thin static light sheet showing high resolution in the center of the field of view.



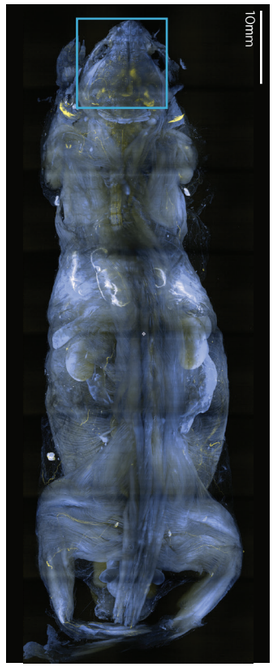
AxL CTLS light sheet with high resolution across the field of view.

Dean, K. M., Roudot, P., Welf, E. S., Danuser, G., & Fiolka, R. (2015). Deconvolution-free Subcellular Imaging with Axially Swept Light Sheet Microscopy. Biophysical journal, 108(12), 2807–2815. https://doi.org/10.1016/j.bpj.2015.05.013

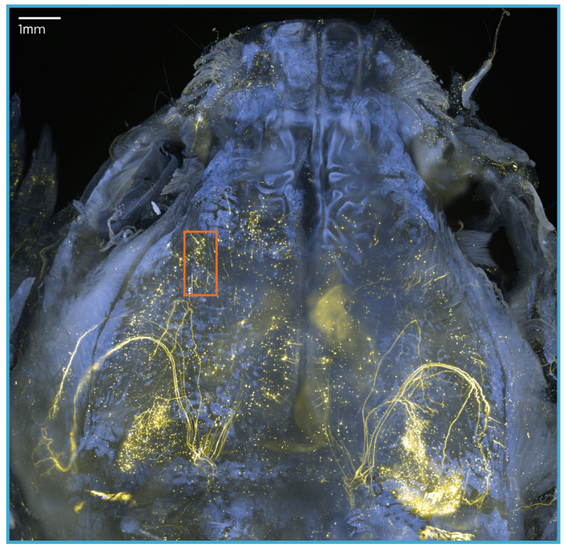
High Speed High Resolution Imaging

The large field of view of AxL CTLS enables ultrafast imaging of whole organs to small animals. An entire mouse can be prescanned in less than 60 seconds, imaged in 20 minutes and high resolution ASLM scanned in 9 hours revealing neuronal connectivity.

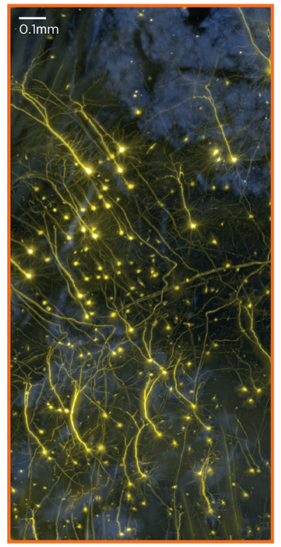
Whole Mouse Scan - 20min



Whole Mouse ASLM Scan - 9hrs



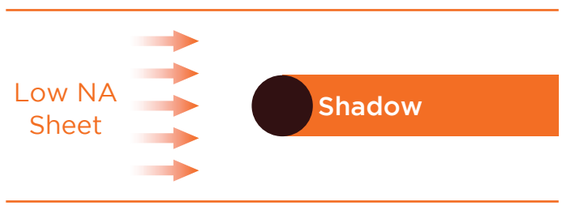
Zoom

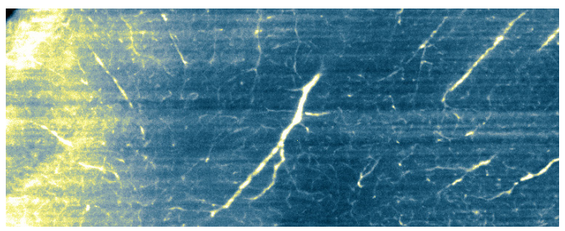


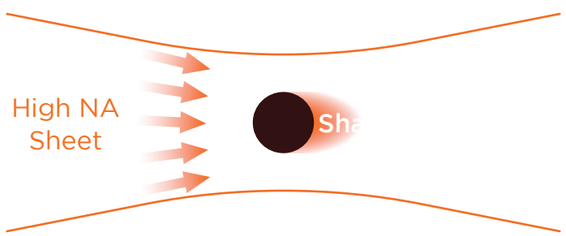
WHOLE MOUSE
Left: Whole mouse imaged in 20min at 0.7x magnification (9.3x9.3x25um voxel size).
Middle: Whole mouse imaged in 9.0h at 1.5x magnification (head displayed only, 4.3x4.3x5um voxel size).
Right: Imaged a subset of the brain at 6.5x magnification (1x1x1um voxel size). Lasers: blue = 488nm, autofluorescence, yellow = 640nm, neurons (Thy1). Courtesy of Dr. Ali Ertürk, Helmholtz Zentrum München.

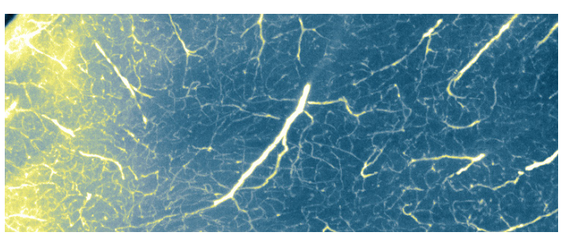
Shadow Reduction

Because ASLM uses the full numerical aperture of the custom excitation objectives, AxL CTLS produces a full distribution of illumination angles minimizing shadow artifacts dramatically.







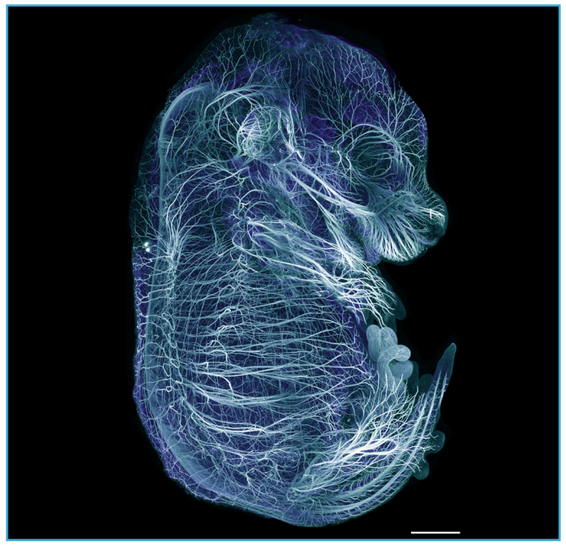


Excitation Objectives

Proprietary AxL CTLS excitation objectives deliver an exceptionally thin lightsheet across a wide 10mm field of view and eliminate spherical aberration at high refractive indices. Coupled with macro zoom imaging objectives, AxL CTLS delivers unparalleled optical sectioning with exceptional imaging quality.

AxL CTLS 0.14NA | RI 1.33-1.56

- Spherical aberration correction for liquid media
- Optimized for clearing solution refractive index
- Diffraction-limited light sheet resolution
- 10mm field of view

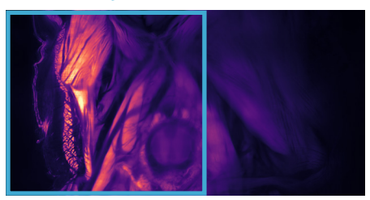


MOUSE EMBRYO
E13.5 mouse embryo cleared with the 3DISCO solvent-based method.
Courtesy of Alain Chédotal, Vision Institute.

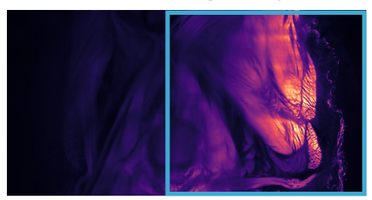
Dual-Sided Illumination

A pair of excitation objectives focus a lightsheet in front of the camera from the left and right. Either left or right side is selected for optimal sheet penetration across wide specimens, resulting in even illumination and neutralization of shadows caused by opaque structures.

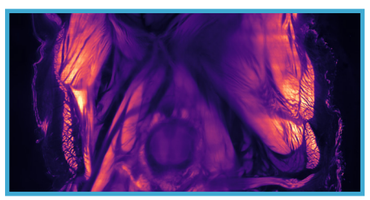
Left Objective



Right Objective

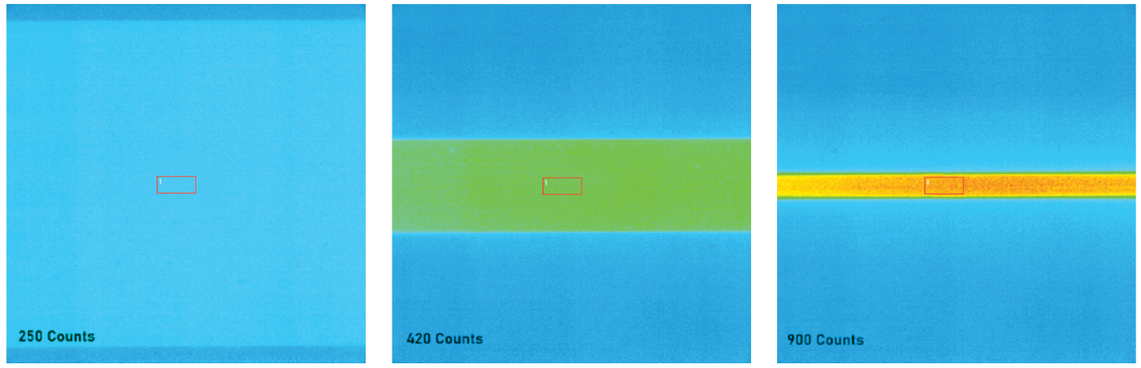


Combined



Digitally Scanned Light Sheet

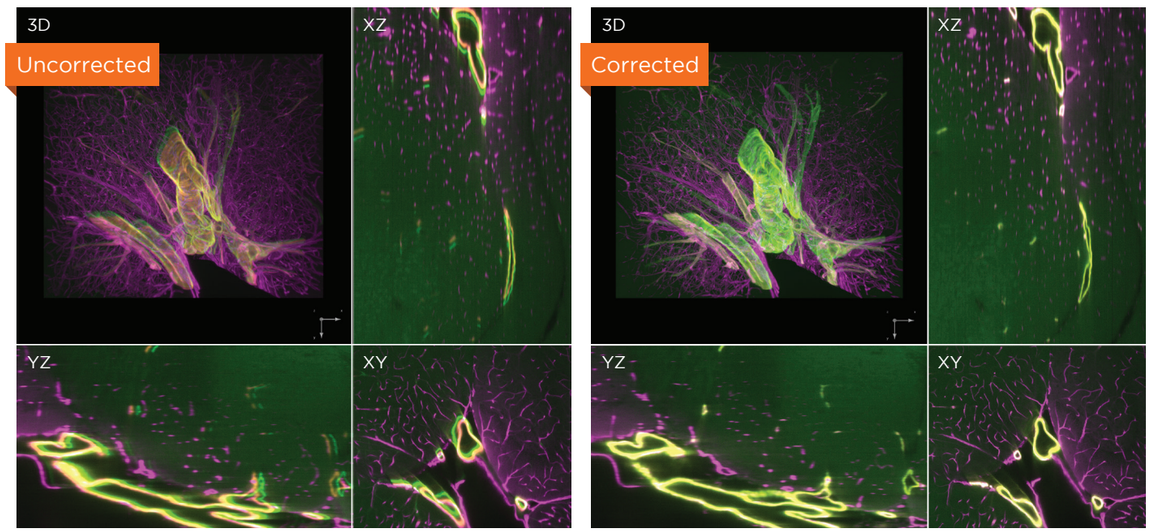
AxL CTLS creates a dynamic lightsheet by scanning a Gaussian laser beam using a high-speed galvo-controlled mirror. This method allows for a tightly focused uniform lightsheet whose width can be seamlessly expanded or contracted to cover various fields of views ranging from 0.5mm to 10mm.



Multichannel Imaging Through Z

AxL CTLS can acquire images of samples maintained in aqueous and solvent-based clearing solutions with refractive indices ranging from 1.33 to 1.56. Changes in refractive index require changes in chromatic correction. AxL CTLS chromatically corrects excitation and detection light during image acquisition across wavelengths. An electrically tunable lens adjusts the light sheet focus and a Z galvo correctly sets the image focal plane on the camera.

Below: Epithelial cells lining the vasculature of a mouse brain imaged by 488nm, 561nm and 640nm lasers. Data are shown in SlideBook's three-view tool showing XZ, YZ and XY perspectives of the gland before and after axial chromatic correction.



SlideBook

SlideBook manages every step in cleared tissue imaging. An intuitive workflow guides users through the collection of 3D stacks, 3D data montaging, volume rendering and finally movie making with story-board support. SlideBook is GPU optimized and readily handles the creation and processing of 3D datasets at over 1TB, making them ready for analysis and rendering. SlideBook SLD files can be accessed via any application supporting Bio-Formats OME, allowing seamless collaboration in any workflow.

User-Selectable App Appearance

Select a color scheme from dozens of options
Switch on-the-fly from dark to light themes

SlideBook Open File Format

Directory-based open file format for big data and high-performance computing applications

Volume Rendering

3D and 4D visualization tools support a user-specified bounding box and a storyboard interface where multiple perspectives can be assembled into a single movie

NVIDIA CUDA GPU Acceleration

GPU acceleration of computationally-intensive operations such as deconvolution

Montage

Montaging of 3D data is built into SlideBook's workflow with spatial and frequency-based algorithms.

Prescan Roadmap

AxL CTLS includes motorized zoom lenses to automatically zoom out and create a 3D map of the entire specimen. This map serves as virtual eyepieces, allowing inspection of the entire specimen at higher magnification and identification of regions of interest for zoomed-in high-resolution 3D imaging.

System Capture Console

The AxL CTLS console is a single easy-to-use window featuring all frequent controls and status displays from laser selection to prescan roadmap to capture.

Capabilities

Capture

Control hundreds of devices including microscopes, stages, lasers, wheels, piezos, scanners, shutters and much more.

View

Visualize data through any numbers of portals, from single images to z-stacks, time lapse, color channels and 4D views.

Analyze

Analyze images and extract statistical data via a wide variety of algorithms while maintaining original data integrity.

Scripting

Macro scripting for capture and analysis enhances the flexibility and power available to users.

Communicate

Present and export data easily as 16-bit TIFFs, 3D movies, graphs or spreadsheets. Data is directly portable to MATLAB and Excel and adheres to Open Microscopy Environment (OME) standards.

Partners

MATLAB

Through hierarchical and conditional capture, user-supplied MATLAB programs can control experimental workflows.

syGlass

syGlass enables 3D and 4D visualization and analysis of SlideBook data in a virtual reality environment.

Aivia

Aivia is an innovative and complete 2D-to-5D image visualization, analysis and interpretation platform with artificial intelligence-guided image analysis.

Dell

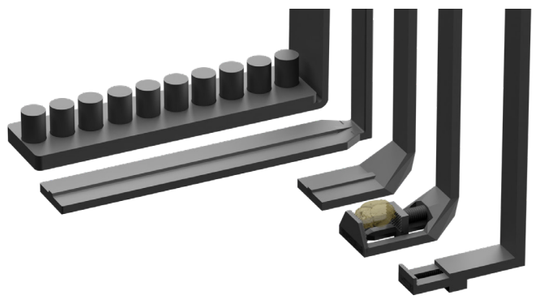
The latest high-power computer workstations control all microscope hardware and enable high-speed processing, segmentation and volume rendering of terabyte (TB) datasets.

Stages, Chambers and Specimen Holders

AxL CTLS incorporates a range of chambers and specimen holders for cleared tissue imaging of whole animals, large organs and smaller specimens. The largest chamber may be used to image an entire mouse or multiple organs while smaller specimens that require a modest amount of index matching media can be imaged in a small, medium or large chamber. High-precision XYZ stages with sub-micron resolution allow for accurate positioning of the specimen. A variety of sample holders optimized for mounting cleared tissue are non-reactive with both aqueous and solvent-based clearing solutions.

Sample Holders

With a range of sizes to choose from, these holders are versatile and adaptable to accommodate various sample types. Sample holders are available in various sizes and types including vise-grip style and magnetic mounts

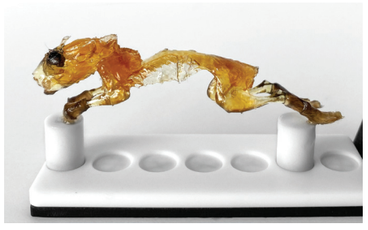


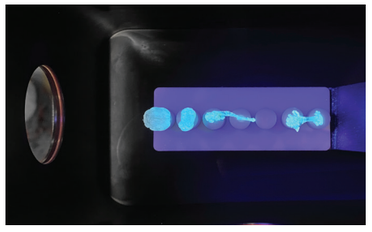
	Small	Medium	Large	XL
Maximum Sample Dimensions (LWH)	10 x 10 x 10mm	25 x 25 x 20mm	50 x 25 x 20mm	100 x 30 x 30mm
Imaging Media Volume	125mL	250mL	500mL	1000mL

Model Organisms

Multi-Specimen Holder

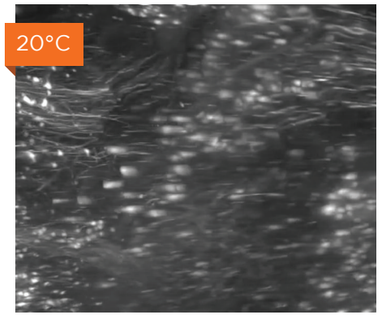
Up to 10 different samples, or a whole cleared animal, can be imaged with the multi-specimen holder. Samples are attached to magnetic pedestals that can be stored in index-matching media until they are ready to be imaged.

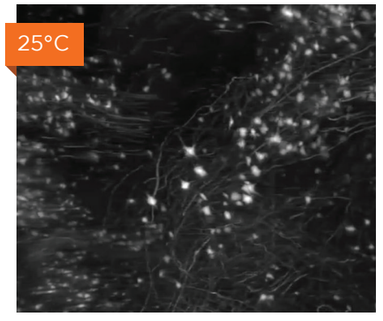




Temperature Controlled Media

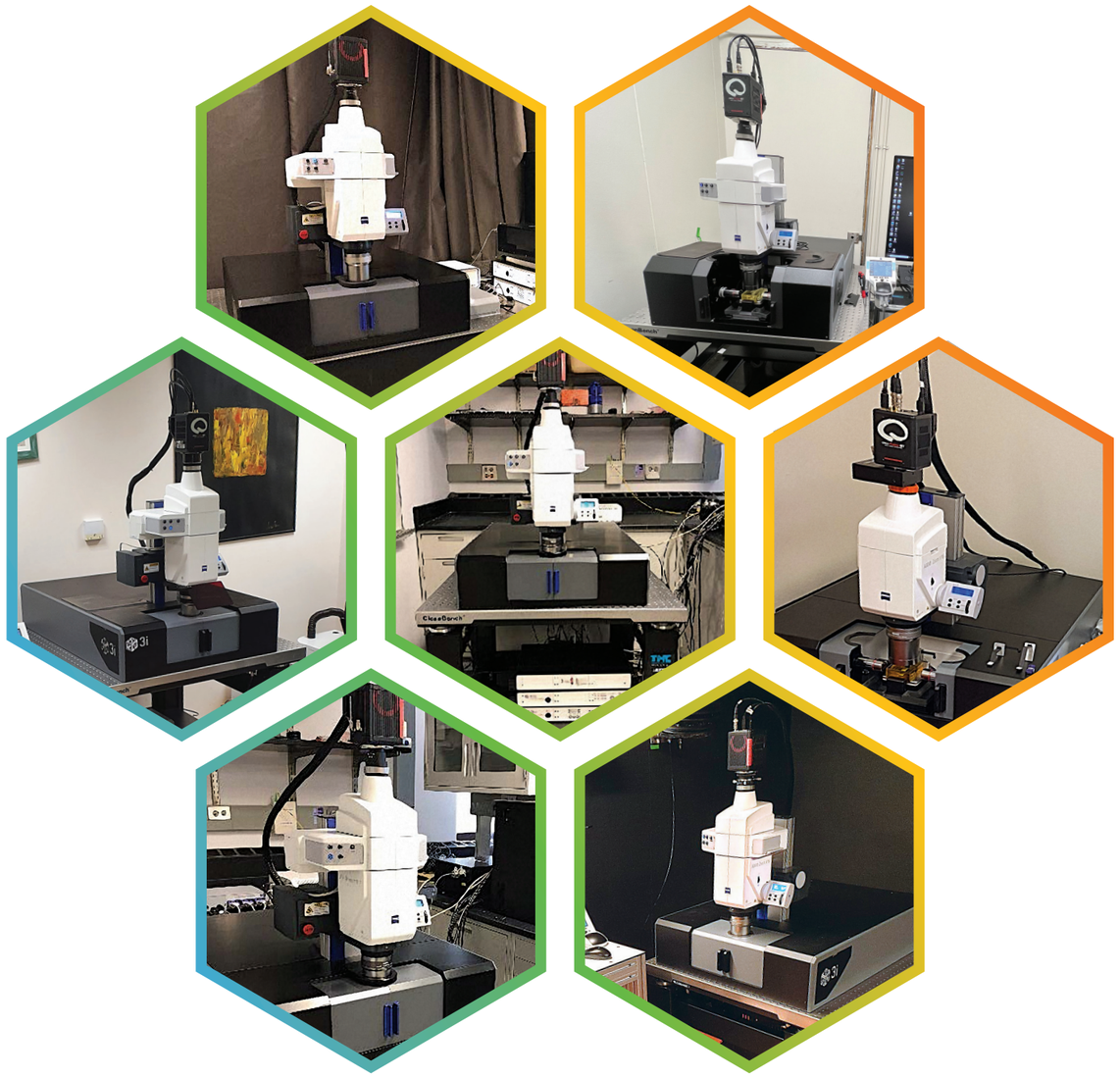
Maintaining constant temperature over time is extremely important to data integrity in multi-hour acquisitions. Small changes in temperature will alter the refractive index of the immersion media, resulting in a shift in the beam above or below the plane of focus. The AxL CTLS specimen chambers are thermally stable to +/- 1ºC.





Systems Engineering

3i's Systems Engineering department designs, builds and extensively tests every customer system. From spinning disk confocal to multiphoton to lightsheet and photomanipulation, 3i has delivered over a thousand custom, cutting-edge microscopy systems to help answer some of the most complex scientific questions.



Application Knowledge | Scientific Consulting

A team of PhD scientists meet with each client to document and better understand the scientific context of the user group to ensure that the capabilities of the delivered system match the underlying research goals.

Performance Criteria | Targeted to Experiments

Understanding key experiments and imaging paradigms allows Systems Engineering to apply targeted testing criteria to every system.

Customized Hardware | Novel Light Creation

No matter how complex or customized a light path may be for imaging or photostimulation, our engineers ensure that light is manipulated and directed to where it is needed, when it is needed.

Custom Test Plan | Assure Experiment Success

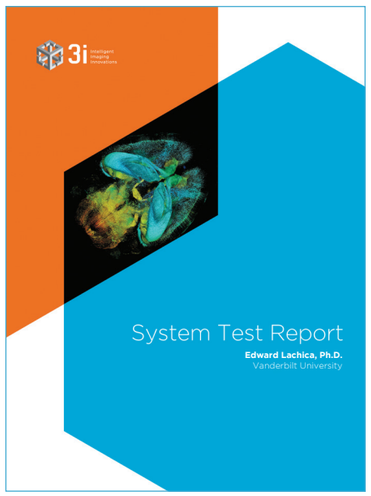
When a technically advanced experiment requires specific system performance to succeed in the lab, a custom test plan assures the system meets that mark prior to delivery.

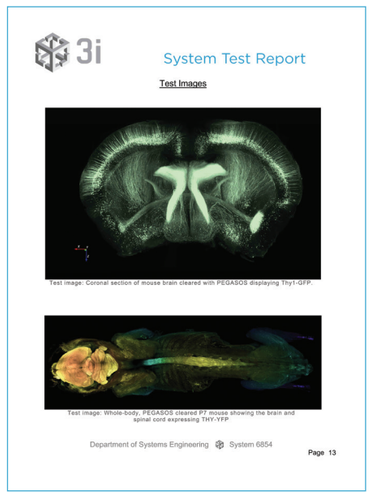
System Integration | Synchronization of Dozens of Instruments

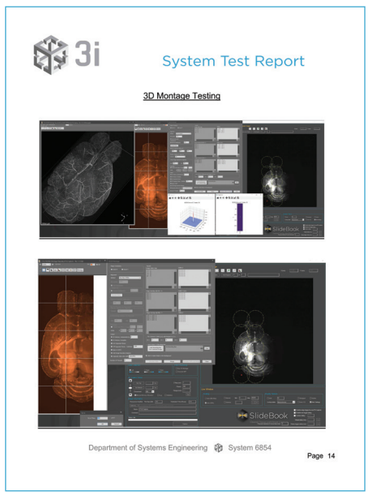
Systems Engineering combines institutional knowledge and scientific consultation to ensure that the instruments in each system are configured for experimental success in the lab.

System Test Report | Guaranteed Performance

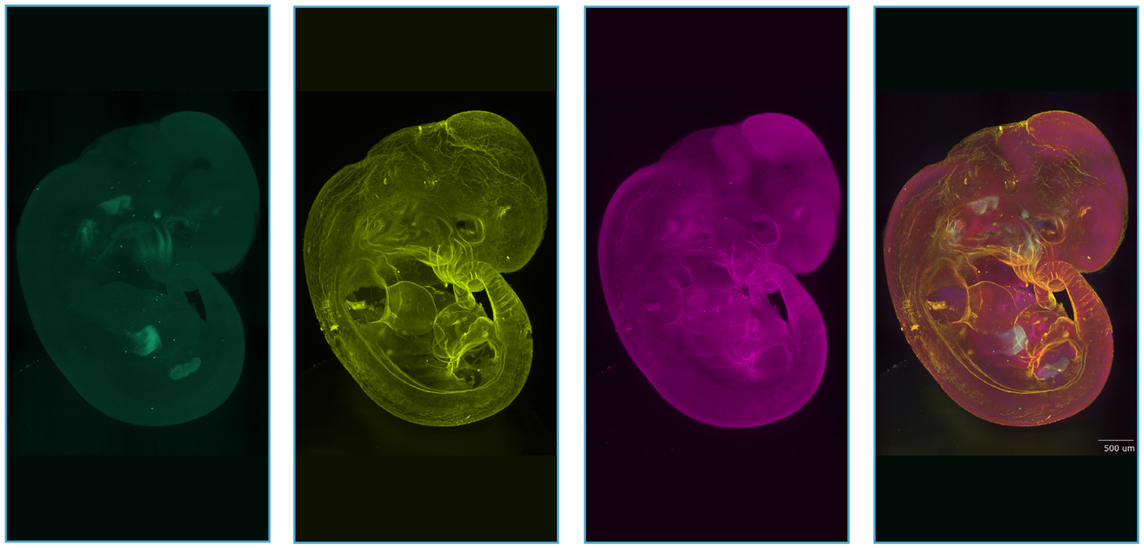
Performance metrics and results of the custom test plan are documented in a System Test Report delivered with each system.



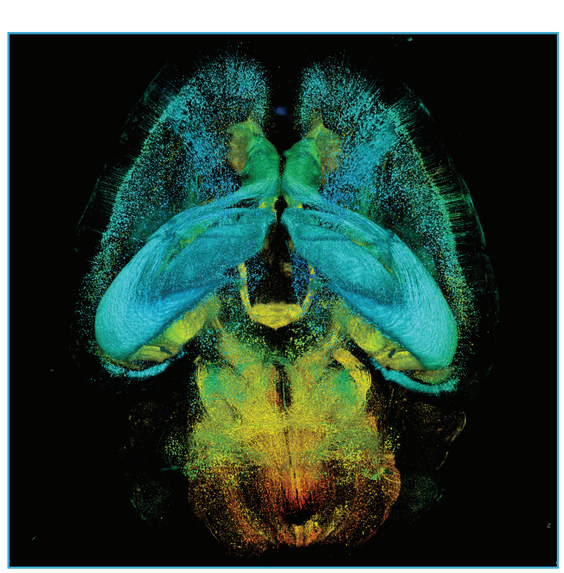




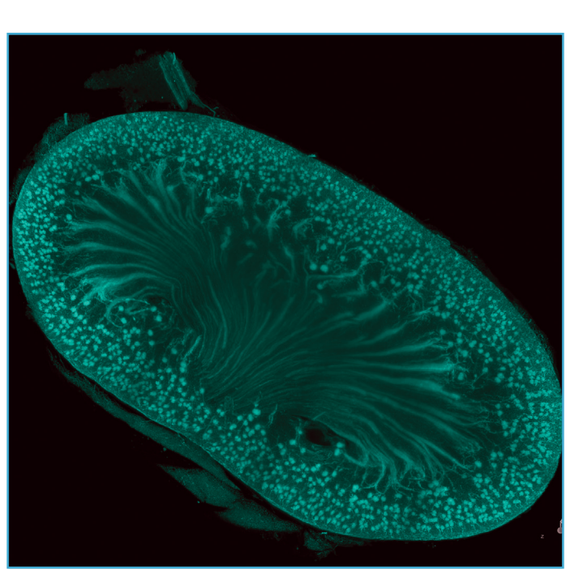
Application Data



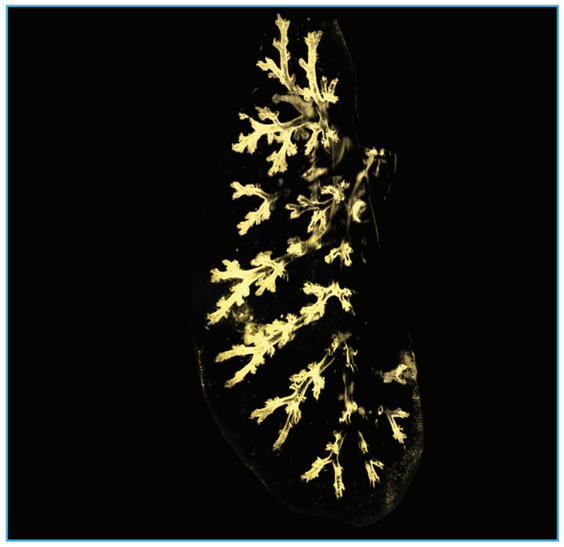
MOUSE EMBRYO
E12 Mouse Embryo cleared with the BABB solvent-based method. Courtesy of Alex Combes and Julie Moreau, Monash University.



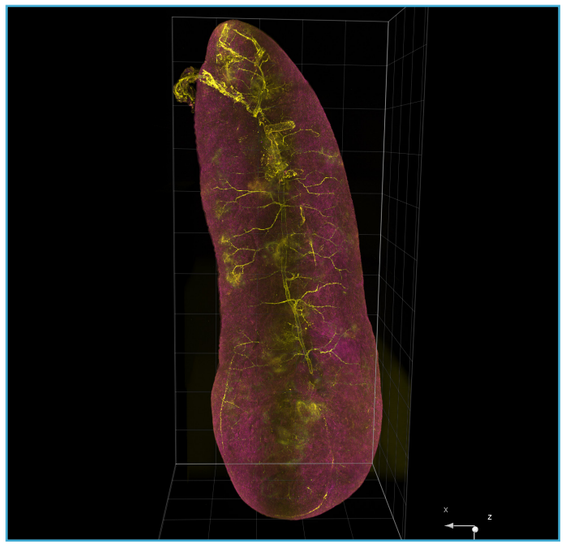
BRAIN
Thy1-GFP mouse brain cleared with the PEGASOS solvent-based method and imaged with the 1.5x / 0.37NA objective in 90 minutes. SlideBook 3D visualization with lookup tables by depth. Courtesy of Dr. H. Zhao, Beijing Institute for Brain Research.



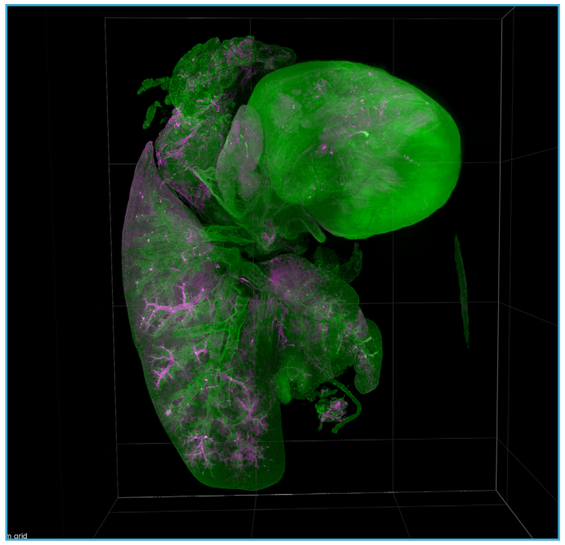
KIDNEY
Glomeruli are shown using td-tomato in a kidney cleared with the PEGASOS solvent-based method. Courtesy of Dr. B. Shen, University of Texas Southwestern.



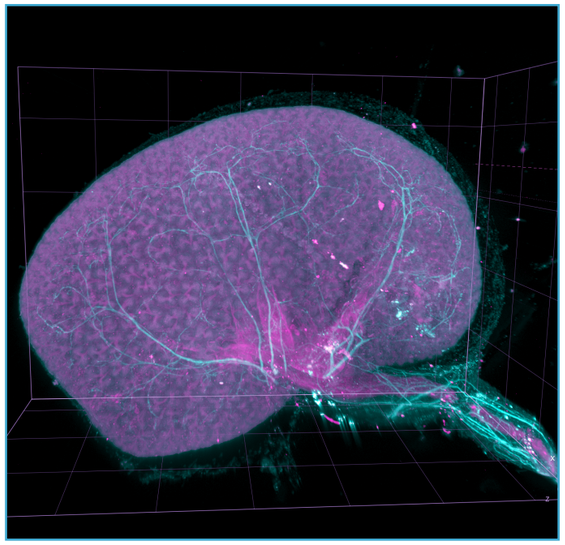
LUNG
Mouse lung cleared with the CUBIC aqueous-based method showing the secondary and tertiary bronchi and bronchioles. Courtesy of Dr. L. Arispe, Northwestern University.



SPLEEN
Mouse spleen cleared with the EZ View aqueous-based method. Courtesy of Juan Cerda, Baylor College of Medicine.

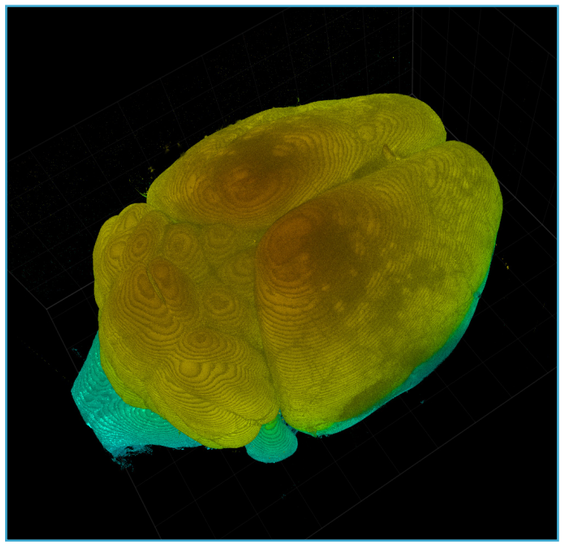


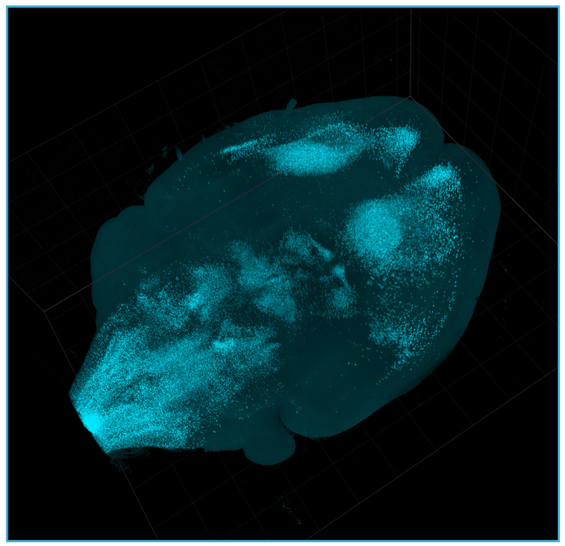
HEART AND LUNG
Heart and lung cleared with the iDISCO solvent-based method. Courtesy of Josh Wythe, University of Virginia.



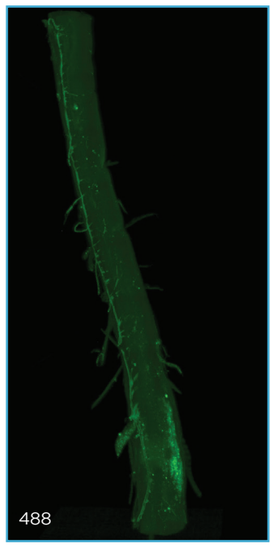
KIDNEY
E18 mouse kidney cleared with the iDISCO solvent-based method. Courtesy of Lori O'Brien, University of North Carolina, Chapel Hill.

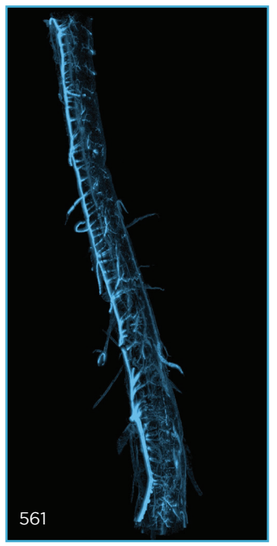
Application Data



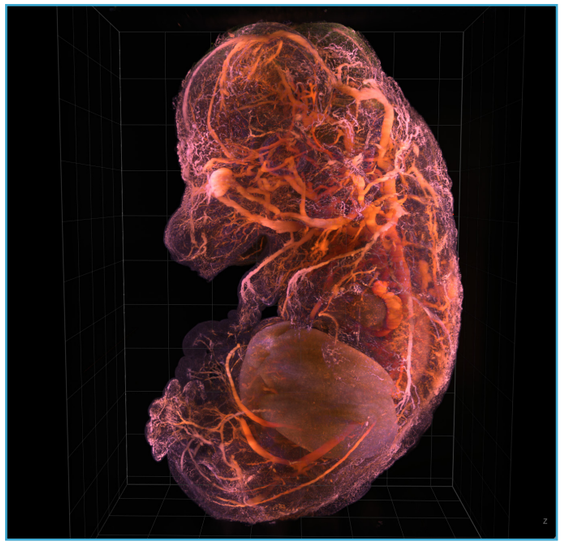


MOUSE BRAIN
Cortico-spinal projections showing neurons in the hypothalamus connecting to the spinal cord. Mouse brain cleared with the 3DISCO solvent-based method. Courtesy of Dr. P. Tsoulfas, University of Miami, Miami Project to Cure Paralysis.

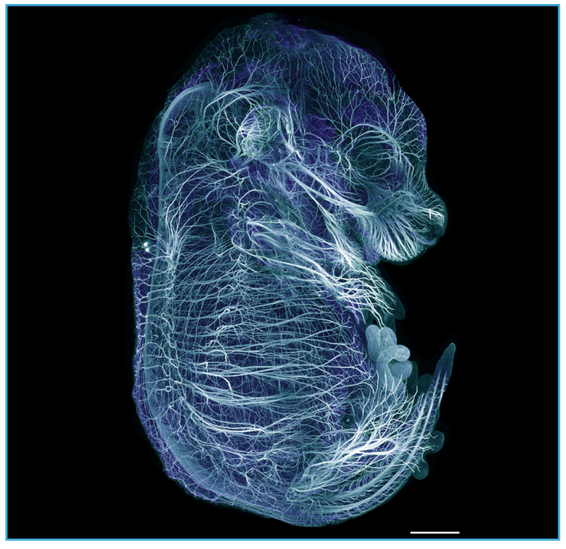




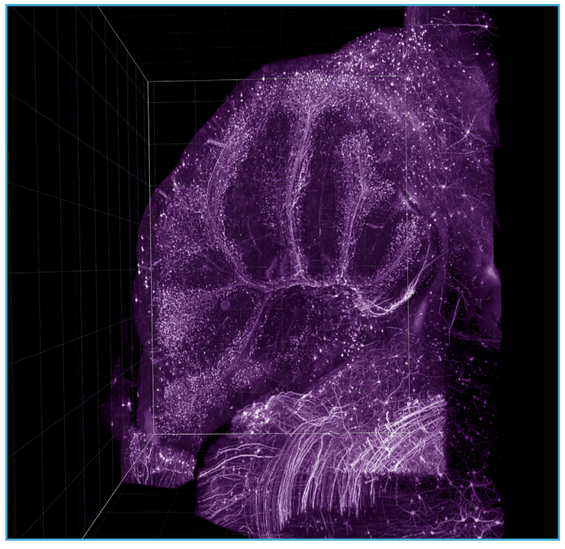
SPINAL CORD
3 different epithelial markers were used to interrogate the vascular morphology of the mouse spinal cord. This specimen is 1.8cm long and cleared with the iDISCO solvent-based method. Courtesy of Dr. P. Tsoulfas, University of Miami, Miami Project to Cure Paralysis.



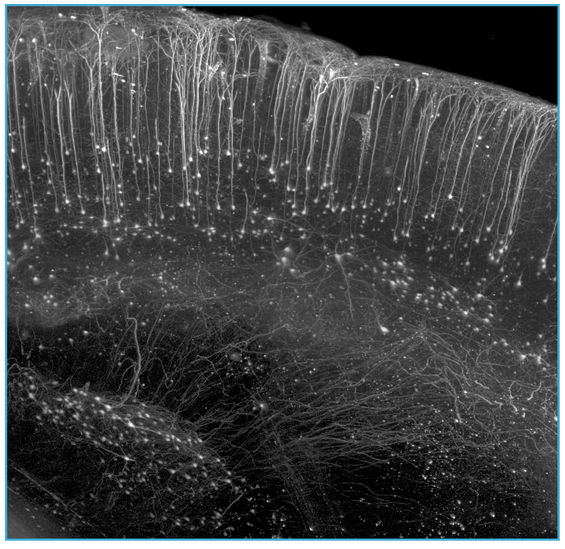
MOUSE EMBRYO
E13 mouse embryo cleared with the 3DISCO solvent-based method. Courtesy of Ted Usdin and David Leopold, National Institutes of Health (NIH).



MOUSE EMBRYO
E13.5 mouse embryo cleared with the 3DISCO solvent-based method. Courtesy of Alain Chédotal, Vision Institute.



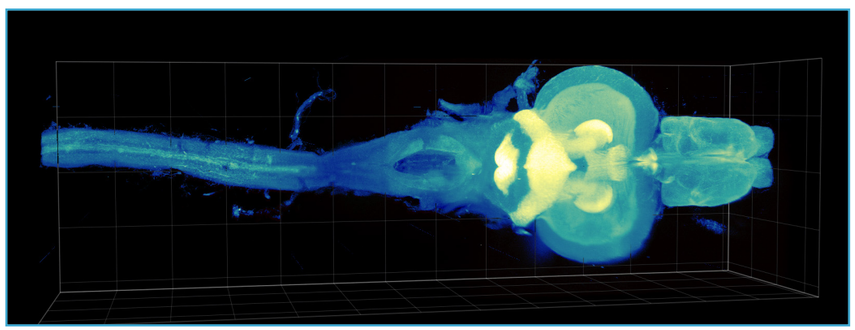
BRAIN
Mouse cerebellum cleared with the iDISCO solvent-based method. Courtesy of Jessica Verpeut, Arizona State University.



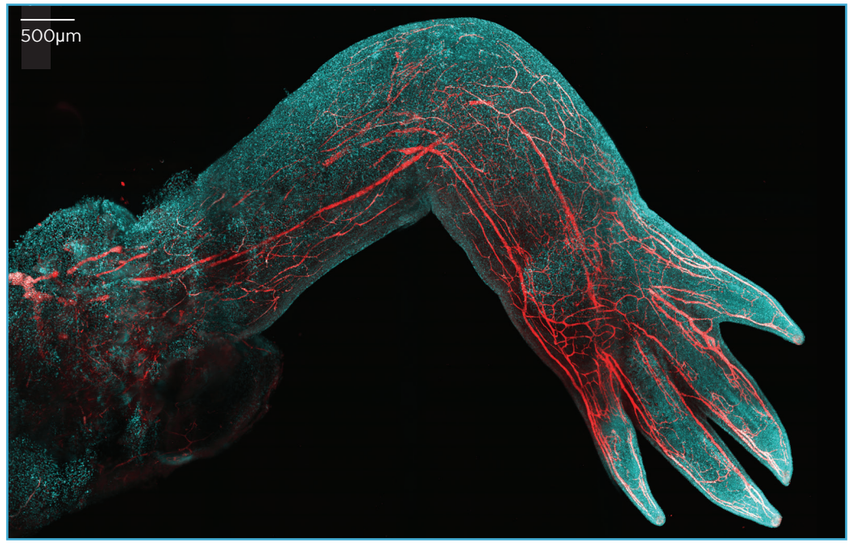
BRAIN
Mouse cortical pyramidal cells cleared with the iDISCO solvent-based method. Courtesy of Jessica Verpeut, Arizona State University.

Application Data

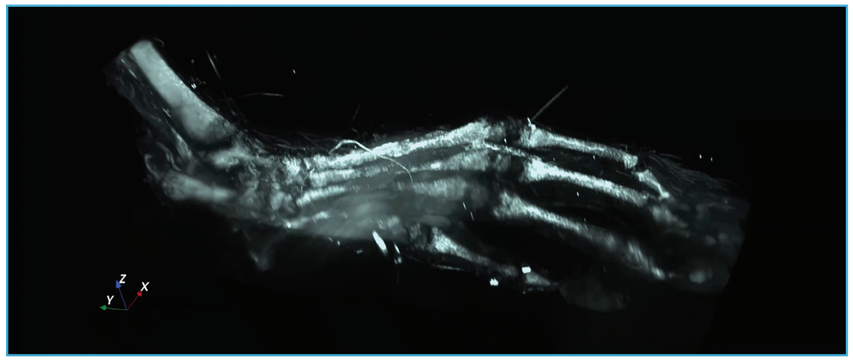
HIND LIMB
Mouse hindlimb with femur and tibia cleared with the EZ View aqueous-based method. Courtesy of Josh Wythe, Gabrielle Largosa and Ted Ahn, University of Virginia.



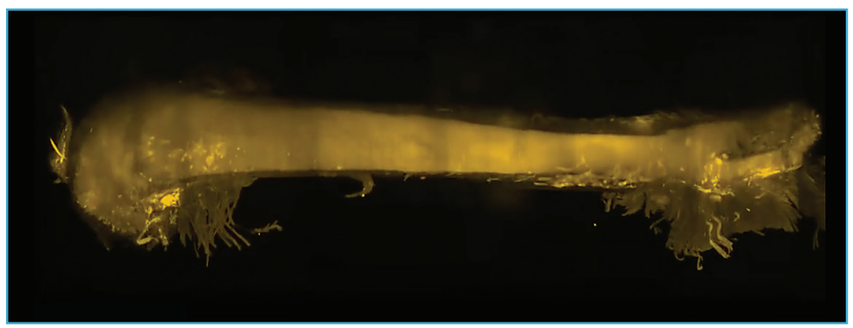
ZEBRAFISH
Whole zebrafish brain cleared with the iDISCO+ solvent-based method. Courtesy of Liz Haynes, The Morgridge Institute for Research.



LIMB
Axolotl limb from the transgenic line CNP-EGFP, which labels the nervous system. Stained for GFP with an Alexa-Fluor 594 secondary antibody, and nuclear staining with Sytox Green. Image courtesy of Maximina Yun Lab, Center for Regenerative Therapies TU Dresden (CRTD). Clearing protocol: Subiran Adrados C, Yu Q, Bolaños Castro LA, Rodríguez Cabrera LA, Yun MH. (2020) Salamander-Eci: An optical clearing protocol for the three-dimensional exploration of regeneration. Developmental Dynamics. https://doi.org/10.1002/dvdy.264



HIND PAW
Mouse hind paw labeled with Thy-1 YFP and cleared with the PEGASOS solvent-based method. Specimen courtesy of Dr. Wenjing Luo, Texas A&M Health Sciences Center.



FEMUR
Mouse femur cleared with the PEGASOS solvent-based method and labeled with Thy1-YFP. Sample courtesy of Dr. Weijing Luo, Texas A&M Health Science Center.

Support and Maintenance

A variety of software and equipment support levels help keep systems running well for years. A Software Support Agreement allows labs to run the latest version of SlideBook with new acquisition and analysis features. It includes direct access to 3i staff via email, phone and video chat. A System Maintenance Agreement adds an annual preventative maintenance visit, 3i service visits and 3i coordination of any repairs, although repair and replacement parts are not included. A System Extended Warranty adds full coverage for repairs and replacement parts. Additionally, 3i application scientists may provide in-person and webinar-based application training.

	Software Maintenance	System Maintenance	System Warranty
Phone, Email and Video Chat Support			
SlideBook Software Releases			
Service Visits and Annual PM Visit			
Repairs Coordinated by 3i			
Application Training | In-Person or Online			
Full Warranty Coverage of all System Hardware			

BUILT BY SCIENTISTS FOR SCIENTISTS

3i designs and manufactures technologies for living cell, live cell, and intravital fluorescence microscopy including superresolution, computer-generated holography, spinning disk confocal, multi-photon and lightsheet. SlideBook software manages everything from instrument control to image capture, processing and data analysis. 3i was established in 1995 by a group of cell biologists, neuroscientists, and computer scientists to provide advanced multi-dimensional microscopy platforms that are intuitive to use, modular in design, and meet the evolving needs of investigators in the biological research community.

3i Intelligent Imaging Innovations

SlideBook

USA
Intelligent Imaging Innovations, Inc.
3575 Ringsby Court, Suite 102
Denver, CO 80216 USA
+1 303 607 9429

UK
Intelligent Imaging Innovations Ltd
17 Westbourne Studios
242 Acklam Rd, London, UK W10 5JJ
+44 2037 440031

DE
Intelligent Imaging Innovations GmbH
Königsallee 9-21
D-37081 Göttingen, Germany
+49 551 508 39 266

FR
Intelligent Imaging Innovations SAS
17 rue Moreau
75012 Paris, France

info@intelligent-imaging.com
sales@intelligent-imaging.com
www.intelligent-imaging.com